

Microbiology of Lebanon Bologna

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Various aspects of the microbiology of the Lebanon bologna process were studied. Manufacture of Lebanon bologna appeared to be similar to that of summer sausage and other fermented sausages and consisted of a lactic acid fermentation by lactobacilli accompanied by the production of cured meat color from the reduction of nitrate by micrococci. The traditional process consists of aging coarse ground beef at 5 C for several days. Aging the beef for about 10 days was necessary to allow development of lactic acid bacteria; for successful fermentation, the concentration of lactic acid producers must be 10⁴/g or more. At least 3% NaCl was necessary to suppress the development of pseudomonads during the aging period; higher concentrations of salt suppress the development of the lactic acid-producing flora. During aging, in the presence of salt, the predominant flora developing on the meat consisted of catalase-positive, gram-positive rods and cocci; during fermentation at 35 C, the predominant flora became catalase-negative, gram-positive rods with characteristics of lactobacilli. Lebanon bologna could be made from frozen beef if the meat was thawed, salted, and aged. However, bolognas could not be made from unaged beef unless a lactic acid starter culture was used. The microflora of several commercial bolognas is reported also.

Lebanon bologna is a semidry, fermented, all-beef sausage which is smoked but not cooked. The sausage originated among the Pennsylvania Germans in the Lebanon, Pa., region. There is a paucity of literature concerning Lebanon bologna technology and microbiology; however, federal specifications for "Lebanon" style bologna have been published (3) and a nontechnical discussion of the processing of Seltzer brand Lebanon bologna has appeared (1). A few spice formulations for Lebanon bologna are known (8, 9, 13). Various aspects of the manufacture of Lebanon bologna have been presented elsewhere (Palumbo et al., manuscript in preparation). This paper is concerned with the microbiology of Lebanon bologna technology.

MATERIALS AND METHODS

Viable counts on meat or Lebanon bolognas were performed as follows: 50 g of beef cubes or 50 g of material from the center of a bologna were removed aseptically and ground at high speed in a Waring blender with 200 ml of peptone water, and appropriate dilutions were then surface plated in triplicate. The types of media employed, temperatures, and times of incubation were: (i) total aerobic count on APT agar, 3 days at 25 C; (ii) micrococci on phenol red mannitol salt agar (MSA), 3 days at 32 C; (iii)

lactic acid bacteria on acidified Rogosa SL agar (RSL), 3 days at 25 C; (iv) yeast on acidified potato dextrose agar (PDA), 3 days at 25 C; and (v) gram-negative bacteria on eosin methylene blue agar (EMB), 1 day at 37 C. All media were obtained from Difco Laboratories, Detroit, Michigan. Dilutions were made in 0.1% peptone (Difco) water. Gram stains of all colony types found on the various media were examined; in addition, the catalase test was performed on isolated colonies.

RESULTS

Viable counts were determined on 14 commercial brands of sausages, including 10 Lebanon bologna types. The viable counts are presented in Table 1 and types of microorganisms are presented in Table 2. The fermented sausages had catalase-negative, gram-positive rods (characteristic of lactobacilli) on APT and RSL except for the products produced by companies D and H, which contained catalase-negative, gram-positive cocci. Company D adds a pediococcus starter culture to their Lebanon bologna, and the predominant flora of thuringer has been shown to be pediococci (2). A number of the sausages contained lactic acid bacteria that produced gum on RSL plates. Lactics were not found in the sweet bologna produced by company C, and this is reflected in the high pH

TABLE 1. Number of viable microorganisms present in selected commercial Lebanon bolognas and other sausages

Company	Type of sausage	Viable counts per g of meat plated on					pH of bologna ^a
		APT	PDA	RSL	EMB	MSA	
A	Regular Lebanon	4.5×10^7	1.9×10^4	3.0×10^6	1.0×10^2	1.8×10^4	4.6
A	Sweet Lebanon ^b	1.9×10^7	$<1 \times 10^2$	1.7×10^7	$<1 \times 10^2$	3.6×10^3	4.9
B	Regular Lebanon	4.4×10^7	$<1 \times 10^2$	1.6×10^7	$<1 \times 10^2$	1.0×10^2	4.6
B	Sweet Lebanon	1.8×10^6	$<1 \times 10^2$	1.5×10^6	$<1 \times 10^2$	1.0×10^2	4.8
C	Regular Lebanon	1.1×10^6	$<1 \times 10^2$	7.5×10^7	3.5×10^3	8.0×10^4	4.7
C	Sweet bologna ^c	4.0×10^4	$<1 \times 10^2$	$<1 \times 10^2$	4.2×10^2	6.0×10^2	5.6
D	Regular Lebanon	2.0×10^6	$<1 \times 10^2$	1.6×10^6	2.0×10^3	4.4×10^3	4.8
D	Sweet Lebanon	1.1×10^6	$<1 \times 10^2$	9.0×10^5	1.1×10^3	3.0×10^2	4.9
F	Regular Lebanon	7.0×10^6	$<1 \times 10^2$	7.5×10^6	1.1×10^3	1.0×10^4	4.6
G	Regular Lebanon	5.3×10^6	$<1 \times 10^2$	2.5×10^6	4.0×10^4	3.0×10^5	4.9
G	Sweet Lebanon	2.1×10^6	1.0×10^2	2.1×10^6	3.4×10^3	8.0×10^4	4.9
H	Thuringer	2.3×10^3	$<1 \times 10^2$	3.0×10^2	$<1 \times 10^2$	2.0×10^2	5.0
I	Dry Italian salami	4.0×10^8	3.2×10^4	1.1×10^8	5.0×10^4	4.4×10^4	5.2
J	Cervelat	1.1×10^8	$<1 \times 10^2$	4.8×10^7	1.3×10^4	6.0×10^2	4.8

^a The pH was determined by inserting the electrode into the mass of meat or into the center of a bologna.

^b A "sweet" Lebanon is similar to the regular type except that more sugar is present. The pH is low but the sweetness masks the acid "tang."

^c The sweet bologna produced by company C was not a Lebanon variety nor was it a fermented product.

of 5.6. Typical coliforms were not observed in any of the sausages. Micrococci were present in company A's sweet Lebanon and in both types of Lebanon bologna produced by company G as well as in the cervelat and dry Italian salami; micrococci were not found in the other sausages.

The individual steps in Lebanon bologna manufacture were investigated to determine the microbial flora involved and the factors responsible for the development of the flora that brings about the desired acid formation and nitrate reduction.

The data in Fig. 1 represent the course, with time, of the viable count in aging salted beef. The viable counts on all five types of media used show a gradual increase in numbers during the aging period. The colony types found on APT consisted of catalase-positive, gram-positive, and gram-negative rods; catalase-positive, gram-positive cocci were found on MSA. The organisms on EMB were catalase-positive, gram-negative rods, but the colonies were not typical coliforms in appearance. Yeasts were present on PDA and catalase-negative, gram-positive rods characteristic of lactobacilli were found on RSL.

The viable counts for the bolognas made from meat aged for varying periods of time are given in Table 3. The only bologna that had a low pH (4.5) was made from meat aged 14 days, at which time the lactic count on RSL was approximately 10^4 /g (Fig. 1). In general, if the lactic acid bacteria count of the salted beef was not in the 10^4 /g range, then a low pH was not achieved in the bolognas. Both APT and RSL plates show catalase-negative, gram-positive rods, with a large number of gum producers on RSL; yeasts tend to disappear in the bolognas (Table 3). Typical coliforms were not observed; MSA contained catalase-positive, gram-positive cocci in all of the bolognas except the one in which the pH was 4.5; at the low pH, the cocci were replaced by catalase-positive, gram-negative rods.

In aging beef for Lebanon bologna manufacture, the level of NaCl used is critical. As the salt concentration is increased, the total aerobic count (APT), lactic acid bacteria count (RSL), and the gram-negative bacterial count (EMB) decreased (Fig. 2). The counts on MSA and PDA were less affected by increasing salt concentrations. In the absence of NaCl, catalase-

positive, gram-negative short rods were the predominant flora on APT, and at 10 days the meat had the strong fruity odor that is associ-

TABLE 2. Cellular types and gram reaction of the microbial flora found in commercial Lebanon bolognas and other sausages

Com- pany	Sausage type	Microbial types found on the following media ^a			
		APT and RSL	Gum production ^b	EMB ^c	MSA
A	Regular Lebanon	1 ^d	-	2	2
A	Sweet Lebanon	1	+		3
B	Regular Lebanon	1	+		2
B	Sweet Lebanon	1	+		2
C	Regular Lebanon	1	+	2, 4	2
C	Sweet bologna ^e	2, 3, 4		2	2
D	Regular Lebanon	5	-	4	2, 4
D	Sweet Lebanon	5	-	2	2
F	Regular Lebanon	1	-	4	6
G	Regular Lebanon	1	+	4	3
G	Sweet Lebanon	1	-	4	3
H	Thuringer	5	-		6
I	Dry Italian salami	1	+	2	3
J	Cervelat	1	+	2, 4	2, 3, 4

^a Yeast was present on PDA only with company A regular Lebanon, G sweet Lebanon, and I dry Italian salami.

^b Gum production was noted on RSL.

^c No typical coliforms were found on EMB.

^d 1, Catalase-negative, gram-positive rods; 2, catalase-positive, gram-negative rods; 3, catalase-positive, gram-positive cocci; 4, catalase-positive, gram-positive rods; 5, catalase-negative, gram-positive cocci; 6, yeast.

^e There was no growth on RSL at 10⁸ dilution.

ated with pseudomonads. A few catalase-positive, gram-positive rods were found at 1% salt, and at 2% there were about equal numbers of catalase-positive, gram-negative and catalase-positive, gram-positive organisms (APT). At 3 and 4% NaCl, very few gram-negative orga-

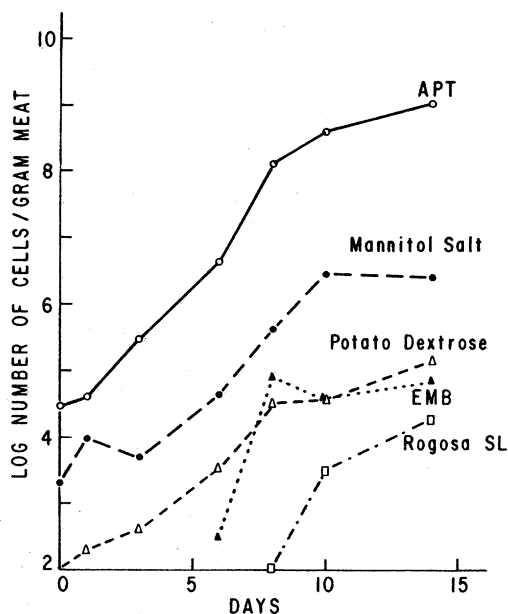


FIG. 1. Influence of time on the viable count of salted beef aging at 5 C. Beef chuck was ground through a 3/4-inch plate, and NaCl was added to the ground meat to make the concentration of salt to 3%. One kilogram of beef cubes was placed into individual plastic bags, and the meat was allowed to age at 5 C. At intervals, a bag was removed from the cooler; 50 g was used to determine the viable count, and the remainder was used to make bolognas. The starting pH of the meat was 5.8 and did not change during the aging period.

TABLE 3. Viable counts of bolognas^a made from meat aged for varying periods of time at 5 C^a

Days ^b	Viable counts per g plated on					pH of bologna
	APT	PDA	RSL	EMB	MSA	
0	1.6 × 10 ⁶	2.0 × 10 ²	5.2 × 10 ⁷	1.3 × 10 ⁴	1.0 × 10 ⁷	5.6
1	4.4 × 10 ⁷	<1 × 10 ²	1.7 × 10 ⁷	3.7 × 10 ³	5.8 × 10 ⁷	5.7
3	2.1 × 10 ⁸	1.0 × 10 ²	1.9 × 10 ⁸	1.5 × 10 ³	1.7 × 10 ⁷	5.8
6	1.4 × 10 ⁸	<1 × 10 ²	1.2 × 10 ⁸	1.0 × 10 ³	2.8 × 10 ⁷	5.4
10	2.1 × 10 ⁸	<1 × 10 ²	1.9 × 10 ⁸	4.0 × 10 ²	4.0 × 10 ⁴	5.2
14	1.2 × 10 ⁹	<1 × 10 ²	2.9 × 10 ⁸	1.7 × 10 ³	1.2 × 10 ⁵	4.5

^a Spices, sugar, and KNO₃ were added to the salted beef cubes and the mixture was ground through a 3/4-inch plate. The mixture was stuffed into casings 55 mm in diameter, and the sausages were incubated at 35 C and 80% RH for 3 days. The sausages were then mellowed at 5 C for 4 days. At the end of the mellowing period, a 50-g sample was removed aseptically from the bologna, and bacterial determinations were made. The viable counts for the meat are given in Fig. 1.

^b Number of days meat was aged at 5 C with 3% NaCl before making bolognas.

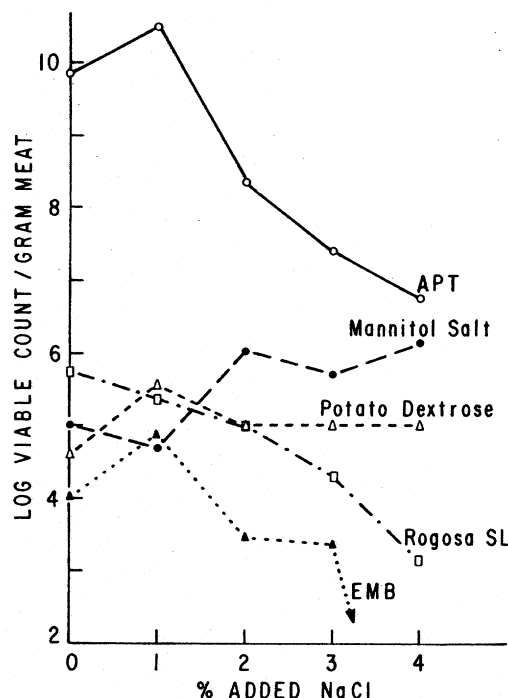


FIG. 2. Effect of varying salt concentrations on the viable count of beef aged for 10 days. Beef chuck was ground through a $\frac{3}{4}$ -inch plate, and 0, 1, 2, 3, or 4% NaCl was added. Each lot of beef was placed into individual plastic bags, and the meat was allowed to age at 5 C for 10 days. At the end of the aging period, 50 g was used to determine the viable count, and the remainder was used to make bolognas. The starting pH of the meat was 5.6 and did not change during the aging period.

nisms were present on APT. The microorganisms found on RSL were catalase-negative, gram-positive rods; catalase-positive, gram-positive cocci were present on MSA, and yeasts were found on PDA.

In Table 4, the viable counts of bolognas prepared from meat aged with varying concentrations of NaCl are presented. The counts on APT and RSL were similar, and the organisms found on both media were catalase-negative, gram-positive rods. Micrococci were not found in the bolognas; the organisms found on MSA were catalase-positive, gram-negative and gram-positive rods. Bolognas prepared from meat aged with low salt had a suitably low pH but they were defective in odor and taste. The bolognas made from meat aged with 4% salt did not reach a low pH because the numbers of the lactic acid bacteria were low (Fig. 2).

Under normal conditions of the manufacture of Lebanon bologna, the fermentation occurs

during the smoking process; however, a satisfactory bologna with low pH and good color can be obtained by incubation of the stuffed sausages in a constant temperature-constant humidity cabinet (35 C and 85% relative humidity [RH]). Some studies were done by utilizing the incubator rather than the smokehouse because of the restrictions associated with the use of the smokehouse. Although bolognas of similar pH, color, and texture were obtained by incubating in the smokehouse or incubator, the sequence of the microbial flora observed differed between the smoked and incubated bolognas. The effect of the presence or absence of smoke on the viable count is illustrated in Fig. 3. The counts on APT, RSL, and MSA decreased markedly during smoking at 35 C and during the subsequent mellowing period at 5 C (Fig. 3A). Both APT and RSL agars contained catalase-negative, gram-positive rods. MSA had colonies that consisted of catalase-positive, gram-positive cocci at the beginning of the fermentation, but by the second day of fermentation the number of micrococci decreased. The cocci were replaced on MSA by catalase-positive, gram-positive and gram-negative rods; by 10 days of mellowing, no micrococci could be detected ($<1 \times 10^2$ /g).

With nonsmoked bolognas (Fig. 3B), the MSA count decreased rapidly during the fermentation period, but the APT and RSL counts were not as severely reduced as under smoked conditions. APT and RSL contained catalase-

TABLE 4. Viable counts of bolognas^a made from meat aged with varying amounts of salt

Concn of NaCl in aged meat (%)	Microbial counts per g of bologna plated on ^b			pH of bolognas
	APT	RSL	MSA	
0	6.2×10^8	2.5×10^8	5.0×10^8	4.7
1	2.9×10^8	2.0×10^8	5.1×10^8	4.8
2	5.3×10^8	4.9×10^8	1.1×10^8	4.6
3	7.8×10^8	9.7×10^8	4.0×10^8	4.9
4	2.4×10^8	1.4×10^8	1.3×10^8	5.6

^a Spices, sugar, KNO₃, and salt (to make the concentration to 3% except for the 4%) were added to the beef cubes, and the mixture was ground through a $\frac{3}{4}$ -inch plate. The material was stuffed into casings 55 mm in diameter, and the sausages were incubated at 35 C and 80% RH for 3 days. The sausages were mellowed at 5 C for 1 day and then a 50-g sample from each bologna was removed aseptically for bacterial determinations. The viable count for the meat is shown in Fig. 2.

^b No microorganisms were detected at 10^2 dilution on PDA and EMB.

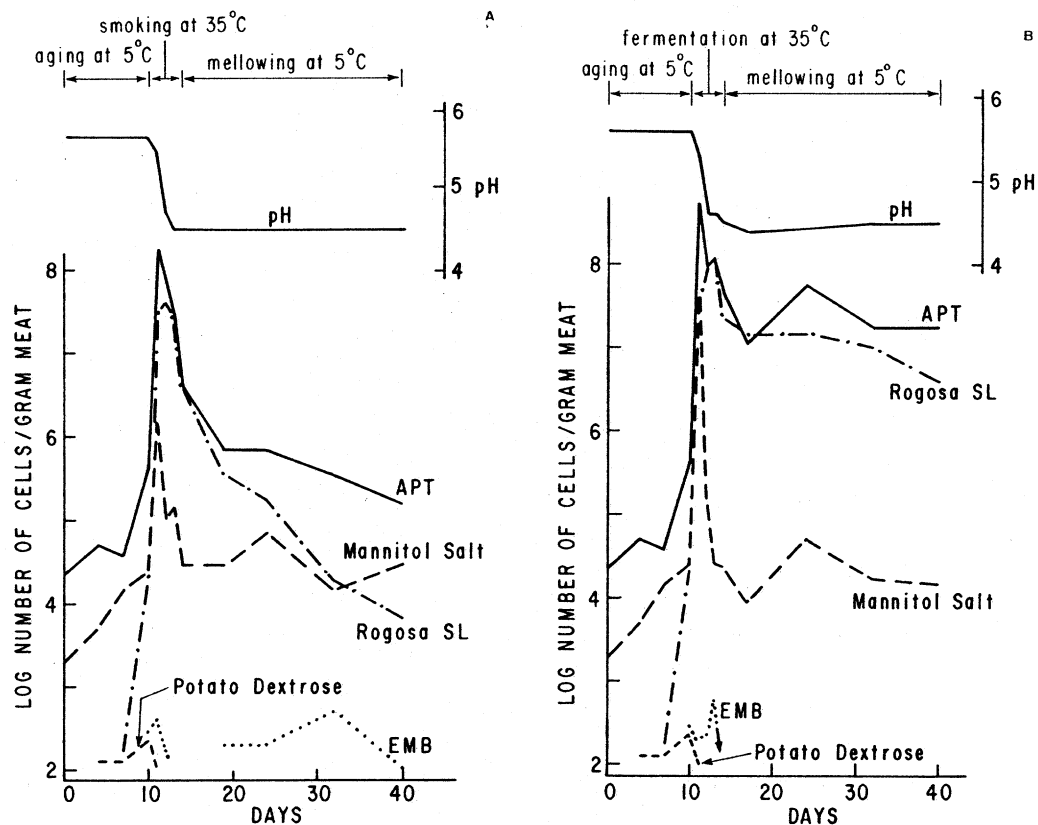


FIG. 3. Changes in the viable count during aging, smoking, and mellowing during Lebanon bologna manufacture. 3A, Smoked; 3B, nonsmoked. Beef chuck was ground through a $\frac{3}{4}$ -inch plate, and NaCl was added to make the concentration of salt to 3%. The salted meat was packed into a wooden barrel and allowed to age at 5 C for 10 days. Spices, sugar, and KNO_3 were added to the meat, and the mixture was

ground through a $\frac{3}{4}$ -inch plate. The material was stuffed into fibrous casings, and the sausages were fermented at 35 C at approximately 90% RH for four days either in an incubator or smoke house. At the end of the fermentation period, the bolognas were mellowed at 5 C. At intervals during the processing, 50-g samples of meat or bolognas were removed for determination of bacterial numbers and pH.

negative, gram-positive rods; MSA showed a rapid die-off of micrococci which were replaced by catalase-positive, gram-negative and gram-positive rods (the microbial pattern on MSA was quite similar in both the smoked and nonsmoked bolognas).

Experiments were performed to determine if Lebanon bologna could be prepared from frozen beef. Viable counts on thawed meat and the bolognas prepared from thawed meat are presented in Table 5. Thawed meat, before the addition of salt, contained catalase-positive, gram-negative rods on APT, yeast on PDA, and catalase-positive, gram-positive cocci on MSA. After 10 days of aging in the presence of salt, the viable count and the types of organisms found in the thawed, salted, aged meat appeared to be similar to that of unfrozen, aged, salted meat:

catalase-positive, gram-positive rods were found on APT, yeast was found on PDA, catalase-negative, gram-positive rods were found on RSL, and catalase-positive, gram-positive cocci were found on MSA. EMB plates had catalase-positive, gram-negative rods which were not typical coliforms. The bolognas made from the thawed beef had a low pH of 4.6 with good color and texture. The organisms found on APT and RSL were catalase-negative, gram-positive rods; catalase-positive, gram-positive and gram-negative rods were found on EMB; MSA had catalase-positive, gram-positive rods and cocci; and no yeast was present at 10^2 dilution on PDA.

Unaged beef or beef aged at 5 C (in the presence of 3% NaCl) for short periods of time did not give Lebanon bologna with a normal low

pH of 4.7 to 4.5 when the fermentation period was 3 to 4 days (Table 3). The fermentation period was lengthened to determine whether bolognas made from fresh meat could attain the desired low pH. The data presented in Table 6 indicate that bolognas made from fresh meat did not reach a normal product pH after 12 days of incubation at 35 C and 80% RH. The microbiology also was different in some respects from the properly fermented product. At days 0, 1, and 2, catalase-positive, gram-positive cocci were the predominant organism on all plates that showed growth except for RSL, which had gum-producing catalase-negative, gram-positive rods. From day 3 on, APT and RSL had catalase-negative, gram-positive rods (gum producing on RSL); catalase-positive, gram-positive cocci were present on EMB and MSA.

A portion of the same beef chuck that was used for the above experiment was ground through a $\frac{3}{4}$ -inch plate, salted (final concentration of NaCl was 3%), and then aged at 5 C for 12 days. The aged meat was made into bolognas in the usual way and incubated at 35 C and 80% RH. The pH of the meat going into the fermentation was 5.3; after 1 day, the pH was 5.3, at 2 days, the pH was 4.7, and at 3 days, the pH was 4.6. Thus, Lebanon bologna could be made from aged beef but not from fresh beef. However, if Lactacel MC (Merck & Co., Rahway, N.J.) starter culture were used with fresh beef and bolognas prepared in the usual manner, a fermented sausage with a pH of approximately 4.5 was produced within 24 h.

DISCUSSION

Semidry and dry sausages are meat products that have been fermented by lactic acid bacteria (2, 10). Our work (Table 1) and the work of others (2; L. B. Jensen and L. S. Paddock, U.S.

Patent 2,225,783,1921) show that lactic acid bacteria are isolated in large numbers from Lebanon bologna.

In the manufacture of Lebanon bologna, cubed beef plus salt is allowed to age at refrigerated temperatures for several days. Pseudomonads and lactic acid bacteria have been shown to be the predominant flora of ground beef during refrigerated storage in the absence of salt (4). At low concentrations, NaCl prevents the growth of pseudomonads (12) but permits the growth of the more salt-tolerant lactic acid bacteria (11). The role of salt as an inhibitor of bacterial growth has been reviewed by Ingram and Kitchell (6). Therefore, the primary reason for salting the cubed beef during the aging

TABLE 6. Preparation of Lebanon bologna from fresh meat^a

No. of days fermentation	Bacterial no. per g of bologna plated on ^b				pH
	APT	RSL	EMB	MSA	
0	2.7×10^3	$<1 \times 10^3$	$<1 \times 10^3$	2.5×10^3	5.3
1	1.7×10^4	3.6×10^3	1.0×10^4	4.5×10^4	5.7
2	1.2×10^7	8.0×10^4	1.0×10^5	5.0×10^4	5.7
3	5.5×10^7	5.3×10^7	1.0×10^8	5.0×10^8	5.7
5	1.2×10^8	9.7×10^7	6.0×10^4	4.0×10^7	5.7
6	8.9×10^7	7.7×10^7	5.0×10^4	5.1×10^7	5.8
7	7.0×10^7	6.0×10^7	5.0×10^4	4.4×10^7	5.4
8	2.7×10^7	1.7×10^7	1.0×10^4	5.0×10^7	5.6
9	1.9×10^7	2.0×10^7	5.0×10^4	3.1×10^7	5.6 ^c

^a Fresh beef chuck was ground through a $\frac{3}{4}$ -inch plate, NaCl, spices, sugar, KNO₃ were added, and the mixture was ground through a $\frac{1}{4}$ -inch plate. The sausage mix was stuffed into casings 55 mm in diameter, and the bolognas were incubated at 35 C and 80% RH. At each time interval, a bologna was removed and bacterial numbers were determined by utilizing a 50-g sample.

^b No growth occurred at 10^2 dilution on PDA.

^c At 12 days, the pH of the bolognas was still 5.6.

TABLE 5. Viable counts of thawed meat and of the bolognas prepared from such meat

Meat	Viable counts determined on					pH
	APT	PDA	RSL	EMB	MSA	
Meat ^a (day 0)	1.3×10^5	6.0×10^2	$<1 \times 10^2$	$<1 \times 10^2$	7.1×10^3	
Salted meat ^b (day 10)	1.1×10^9	8.7×10^4	1.4×10^4	5.8×10^6	9.1×10^5	5.6
Bolognas ^c	1.1×10^9	$<1 \times 10^2$	4.0×10^6	1.2×10^3	2.6×10^5	4.6

^a Beef chuck was ground through a $\frac{3}{4}$ -inch plate, placed in plastic bags, and frozen for approximately 4 months. The meat was thawed for approximately 24 h at 5 C and a 50 g sample was removed to determine bacterial numbers. NaCl was added to make the concentration to 3% salt and a kilogram of salted meat was placed into individual plastic bags and was allowed to age at 5 C.

^b Viable counts were determined after 10 days of aging at 5 C.

^c Spices, sugar, and KNO₃ were added to the salted beef, and the mixture was ground through a $\frac{1}{4}$ -inch plate. The mixture was stuffed into casings 55 mm in diameter, and the sausages were incubated at 35 C and 80% RH for 3 days. The bolognas were mellowed for 4 days at 5 C; bacterial numbers were determined on a 50-g sample.

period in the manufacture of Lebanon bologna is to prevent the growth of the undesirable pseudomonads.

During the aging or holding period used in these studies, microbial populations changed considerably. Deibel et al. (2), in the manufacture of summer sausage, found little or no change in the microbial flora during a short, 2- to 4-day aging period. We found that such a short holding period was not sufficient to permit development of the lactic acid-producing flora. During the long aging period, at least 10 days in our studies, the lactic acid bacteria increased to about 10^4 /g, and this concentration appears to be critical for adequate decrease in pH in the bolognas (Fig. 1; Table 3). Therefore, a long aging period is necessary to allow the development of sufficient numbers of lactic acid bacteria.

Another important function of aging is to allow the micrococci to develop. The micrococci reduce nitrate to nitrite and thus give bolognas a good cured meat color (F. W. Kurk, U.S. Patent 1,380,068, 1921; reference 10). The concentration of micrococci does not seem to be as critical as the concentration of lactic acid bacteria; good color was generally found in all bolognas regardless of the micrococcal count. Micrococci are more halotolerant than lactic acid bacteria and pseudomonads (12).

With too much salt, the increase in the lactic acid bacterial population was very slow and the numbers at the end of the aging period were not high enough to produce bolognas of desirable low pH (data for 4% salt in Fig. 2 and Table 4).

When meat was aged with low concentrations of salt (1-2%), pH decreased satisfactorily during processing but the excessive development of pseudomonads in the meat gave the bologna inferior flavor. Salt at the 3% level appears to be a good compromise between the concentration that inhibits the pseudomonads and one that is not too inhibitory to the lactic acid bacteria.

Our results indicated that smoking is inhibitory to the lactic acid bacteria because the viable count of the lactic flora decreased drastically on both APT and RSL (compare A and B, Fig. 3). Apparently some component of smoke and not acid caused the killing because the pH decrease was similar under smoked and non-smoked conditions. Handford and Gibbs (5), using liquid smoke, found that certain lactic acid bacteria and micrococci were inhibited by smoke constituents.

The MSA count rapidly decreased during fermentation and mellowing under both smoke and nonsmoke conditions (compare A and B, Fig. 3). The loss of micrococci probably was

due to the acid content of the sausage rather than to smoke. Also, the organisms might be sensitive to the nitrite formed by their reduction of nitrate. By day 20, few if any micrococci were found (Fig. 3); they apparently were superseded by the outgrowth of rod forms. The counts on PDA and EMB also decreased during fermentation and mellowing regardless of smoking or nonsmoking. The yeast and gram-negative bacteria are probably sensitive to the acid environment of the sausages.

Sausage makers generally believe that the use of frozen meat does not produce a high-quality product (7). However, our experiments indicate that frozen beef chuck, when thawed, salted, and aged, yields a satisfactory Lebanon bologna.

Prolonged incubation under fermentation conditions did not give a bologna of low pH when fresh rather than aged meat was used (Table 6). However, a portion of the same batch of meat that had been salted and aged at 5 C for 12 days did yield bolognas that reached a low pH in 2 to 3 days. One possible explanation for the failure of the fresh meat bolognas to ferment is that the lactic acid bacteria count never reached a critical level to initiate sufficient acid production. Aging at low temperatures may allow the selection and growth of salt-tolerant organisms that are active acid producers.

In a typical Lebanon bologna process, the micrococci die off quickly once the fermentation leads to acid conditions, but in the bolognas made from fresh beef, the micrococcal count remained quite high (Table 6). When the number of lactic acid bacteria going into the fermentation is low, the continued high density of micrococci might interfere with lactobacilli proliferation by competing for nutrients. Apparently, whatever the reasons, Lebanon bologna of consistently good quality cannot be made from unaged meat. However, fresh meat can be used for Lebanon bologna manufacture if a suitable starter culture is used.

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